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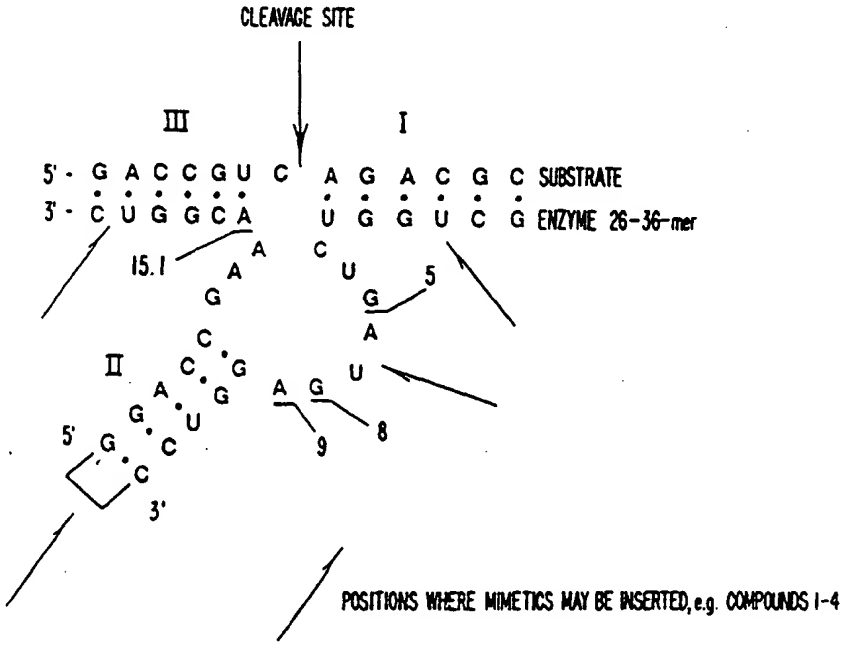
(54) Title: NON-NUCLEOTIDE CONTAINING ENZYMATIC NUCLEIC ACID



(57) Abstract

Enzymatic nucleic acid molecule containing one or more non-nucleotide mimetics, and having activity to cleave an RNA or DNA molecule.

DESCRIPTION

NON-NUCLEOTIDE CONTAINING ENZYMATIC NUCLEIC ACID

Background of the Invention

This application is a continuation-in-part of Usman et al., U.S. Serial No. 08/152,481, filed November 12, 1993 which is a continuation-in-part of Usman, U.S. Serial No. 08/116,177, filed September 2, 1993, both entitled "Non-Nucleotide Containing Enzymatic Nucleic Acid" both hereby incorporated by reference herein (including drawings).

This invention relates to chemically synthesized non-nucleotide-containing enzymatic nucleic acid.

The following is a brief history of the discovery and activity of enzymatic RNA molecules or ribozymes. This history is not meant to be complete but is provided only for understanding of the invention that follows. This summary is not an admission that all of the work described below is prior art to the claimed invention.

Prior to the 1970s it was thought that all genes were direct linear representations of the proteins that they encoded. This simplistic view implied that all genes were like ticker tape messages, with each triplet of DNA "letters" representing one protein "word" in the translation. Protein synthesis occurred by first transcribing a gene from DNA into RNA (letter for letter) and then translating the RNA into protein (three letters at a time). In the mid 1970s it was discovered that some genes were not exact, linear representations of the proteins that they encode. These genes were found to contain interruptions in the coding sequence which were removed from, or "spliced out" of, the RNA before it became translated into protein. These interruptions in

Also in 1986, Cech showed that the RNA substrate sequence recognized by the *Tetrahymena* ribozyme could be changed by altering a sequence within the ribozyme itself. This property has led to the development of a number of site-specific ribozymes that have been individually designed to cleave at other RNA sequences.

The *Tetrahymena* intron is the most well-studied of what is now recognized as a large class of introns, Group I introns. The overall folded structure, including several sequence elements, is conserved among the Group I introns, as is the general mechanism of splicing. Like the *Tetrahymena* intron, some members of this class are catalytic, *i.e.*, the intron itself is capable of the self-splicing reaction. Other Group I introns require additional (protein) factors, presumably to help the intron fold into and/or maintain its active structure.

Ribonuclease P (RNaseP) is an enzyme comprised of both RNA and protein components which are responsible for converting precursor tRNA molecules into their final form by trimming extra RNA off one of their ends. RNaseP activity has been found in all organisms tested. Sidney Altman and his colleagues showed that the RNA component of RNaseP is essential for its processing activity; however, they also showed that the protein component also was required for processing under their experimental conditions. After Cech's discovery of self-splicing by the *Tetrahymena* intron, the requirement for both protein and RNA components in RNaseP was reexamined. In 1983, Altman and Pace showed that the RNA was the enzymatic component of the RNaseP complex. This demonstrated that an RNA molecule was capable of acting as a true enzyme, processing numerous tRNA molecules without itself undergoing any change.

The folded structure of RNaseP RNA has been determined, and while the sequence is not strictly conserved between RNAs from different organisms, this higher order structure is. It is thought that the protein

ribozyme) and only a required UH where H = C, A, or U in the strand that did get cut (the substrate). This resulted in a catalytic ribozyme that could be designed to cleave any UH RNA sequence embedded within a longer "substrate recognition" sequence. The specific cleavage of a long mRNA, in a predictable manner using several such hammerhead ribozymes, was reported in 1988.

One plant pathogen RNA (from the negative strand of the tobacco ringspot virus) undergoes self-cleavage but cannot be folded into the consensus hammerhead structure described above. Bruening and colleagues have independently identified a 50-nucleotide catalytic domain for this RNA. In 1990, Hampel and Tritz succeeded in dividing the catalytic domain into two parts that could act as substrate and ribozyme in a multiple-turnover, cutting reaction. As with the hammerhead ribozyme, the catalytic portion contains most of the sequences required for catalytic activity, while only a short sequence (GUC in this case) is required in the target. Hampel and Tritz described the folded structure of this RNA as consisting of a single hairpin and coined the term "hairpin" ribozyme (Bruening and colleagues use the term "paperclip" for this ribozyme motif). Continuing experiments suggest an increasing number of similarities between the hairpin and hammerhead ribozymes in respect to both binding of target RNA and mechanism of cleavage.

Hepatitis Delta Virus (HDV) is a virus whose genome consists of single-stranded RNA. A small region (about 80 nucleotides) in both the genomic RNA, and in the complementary anti-genomic RNA, is sufficient to support self-cleavage. In 1991, Been and Perrotta proposed a secondary structure for the HDV RNAs that is conserved between the genomic and anti-genomic RNAs and is necessary for catalytic activity. Separation of the HDV RNA into "ribozyme" and "substrate" portions has recently been achieved by Been. Been has also succe ded in reducing the size of the HDV ribozyme to about 60 nucleotides.

virus) which uses RNA as the infectious agent. (Figure 2)

Hepatitis Delta Virus (HDV) Ribozyme

Size: ~60 nucleotides (at present).

Cleavage of target RNAs recently demonstrated.

Sequence requirements not fully determined.

Binding sites and structural requirements not fully determined, although no sequences 5′ of cleavage site are required.

Only 1 known member of this class. Found in human HDV. (Figure 3)

Eckstein *et al.*, International Publication No. WO 92/07065; Perrault *et al.*, Nature **1990**, *344*:565; Pieken *et al.*, Science **1991**, *253*:314; Usman and Cedergren, *Trends in Biochem. Sci.* **1992**, *17*:334; Usman *et al.*, International Publication No. WO 93/15187; and Rossi *et al.*, International Publication No. WO 91/03162, describe various chemical modifications that can be made to the sugar moieties of enzymatic nucleic acid molecules.

Summary of the Invention

This invention concerns the use of non-nucleotide molecules as spacer elements at the base of double-stranded nucleic acid (*e.g.*, RNA or DNA) stems (duplex stems) or in the single-stranded regions, catalytic core, loops, or recognition arms of enzymatic nucleic acids. Duplex stems are ubiquitous structural elements in enzymatic RNA molecules. To facilitate the synthesis of such stems, which are usually connected via single-stranded nucleotide chains, a base or base-pair mimetic may be used to reduce the nucleotide requirement in the synthesis of such molecules, and to confer nuclease resistance (since they are non-nucleic acid components). This also applies to both the catalytic core and recognition arms of a ribozyme.

Examples of such non-nucleotide mimetics are shown in Figure 4 and their incorporation into hammerhead

adenosine, guanine, cytosine, uracil or thymine. It may have substitutions for a 2′ or 3′ H or OH as described in the art. See Eckstein et al. and Usman et al., supra.

In preferred embodiments, the enzymatic nucleic acid includes one or more stretches of RNA, which provide the enzymatic activity of the molecule, linked to the non-nucleotide moiety. The necessary RNA components are known in the art, see, e.g., Usman, *supra*.

As the term is used in this application, non-nucleotide-containing enzymatic nucleic acid means a nucleic acid molecule that contains at least one non-nucleotide component which replaces a portion of a ribozyme, *e.g.*, but not limited to, a double-stranded stem, a single-stranded "catalytic core" sequence, a single-stranded loop or a single-stranded recognition sequence. These molecules are able to cleave (preferably, repeatedly cleave) separate RNA or DNA molecules in a nucleotide base sequence specific manner. Such molecules can also act to cleave intramolecularly if that is desired. Such enzymatic molecules can be targeted to virtually any RNA transcript. Such molecules also include nucleic acid molecules having a 3′ or 5′ non-nucleotide, useful as a capping group to prevent exonuclease digestion.

Enzymatic molecules of this invention act by first binding to a target RNA or DNA. Such binding occurs through the target binding portion of the enzyme which is held in close proximity to an enzymatic portion of molecule that acts to cleave the target RNA or DNA. Thus, the molecule first recognizes and then binds a target nucleic acid through complementary base-pairing, and once bound to the correct site, acts enzymatically to cut the target. Strategic cleavage of such a target will destroy its ability to direct synthesis of an encoded protein. After an enzyme of this invention has bound and cleaved its target it is released from that target to search for

percent complementarity is preferred, but complementarity as low as 50-75% may also be useful in this invention.

In preferred embodiments of this invention, the enzyme molecule is formed generally in a hammerhead motif, but may also be formed in the motif of a hairpin, hepatitis delta virus, group I intron or RNaseP RNA (in association with an RNA guide sequence). Examples of such hammerhead motifs are described by Rossi *et al.*, *Aids Research and Human Retroviruses* **1992**, *8*:183; of hairpin motifs by Hampel *et al.*, "RNA Catalyst for Cleaving Specific RNA Sequences," filed September 20, 1989, which is a continuation-in-part of U.S. Serial No. 07/247,100 filed September 20, 1988, Hampel and Tritz, *Biochemistry* **1989**, *28*:4929, and Hampel *et al.*, *Nucleic Acids Research* **1990**, *18*:299; and an example of the hepatitis delta virus motif is described by Perrotta and Been, *Biochemistry* **1992**, *31*:16; of the RNaseP motif by Guerrier-Takada *et al.*, *Cell* **1983**, *35*:849; and of the Group I intron by Cech *et al.*, U.S. Patent 4,987,071. These specific motifs are not limiting in the invention and those skilled in the art will recognize that all that is important in an enzyme molecule of this invention is that it have at least one non-nucleotide portion, and a specific substrate-binding site which is complementary to one or more of the target gene RNA regions, and that it have nucleotide sequences within or surrounding that substrate-binding site which impart a nucleic acid cleaving activity to the molecule.

The invention provides a method for producing a class of enzymatic cleaving agents which exhibit a high degree of specificity for the nucleic acid of a desired target. The enzyme molecule is preferably targeted to a highly conserved sequence region of a target such that specific treatment of a disease or condition can be provided with a single enzyme. Such enzyme molecules can be delivered exogenously to specific cells as required. In the preferred hammerhead motif the small size (less than 60 nucleotides, preferably between 30-40 nucleotides

compounds 4 and 5 each R may independently be H, OH, protected OH, O-alkyl, alkenyl or alkynyl or alkyl, alkenyl or alkynyl of 1-10 carbon atoms.

Figure 5 is a diagrammatic representation of the preferred location for incorporation of various non-nucleotide mimetics into nucleic acid enzymes. Specifically, mimetics, **1-10**, may replace the loop (denoted as / / in Figure 5) that connects the two strands of Stem II. Stem II itself may be from 1 to 10 base pairs. In examples **1** & **2** below compounds **1** and **2** were incorporated into molecules having a stem II of 1 to 5 basepairs in length. Compounds **1**, **4** and **5** may also replace nucleotides in the recognition arms of stems I and III or in stem II itself.

Figure 6 is a diagrammatic representation of the synthesis of a perylene based non-nucleotide mimetic phosphoramidite 3.

Figure 7 is a diagrammatic representation of the synthesis of an abasic deoxyribose or ribose non-nucleotide mimetic phosphoramidite.

Figures 8a and 8b are graphical representations of cleavage of substrate by various ribozymes at 8nM, or 40 nM, respectively.

Non-nucleotide Mimetics

Non-nucleotide mimetics useful in this invention are generally described above. Those in the art will recognize that these mimetics can be incorporated into an enzymatic molecule by standard techniques at any desired location. Suitable choices can be made by standard experiments to determine the best location, *e.g.*, by synthesis of the molecule and testing of its enzymatic activity. The optimum molecule will contain the known ribonucleotides needed for enzymatic activity, and will have non-nucleotides which change the structure of the molecule in the least way possible. What is desired is that several nucleotides can be substituted by one non-nucleotide to save synthetic steps in enzymatic molecule

In another example, a specific linker for the base of the stem II C-G of a hammerhead ribozyme was designed. Applicant believes that the distance between the C1′ carbons of the C-G base pair is about 16 Angstroms. To join these two pieces of RNA by a covalent analog of the C-G base pair a new type of dimer phosphoramidite containing a linker between the 3′-OH and the 5′-OH of the G and C residues respectively can be constructed. Two types of base-pair mimetic are the rigid aromatic spacers, **2** or **3**, shown in Figure 4. These have been incorporated at the base of stem II of a hammerhead ribozyme as described in Example 1, replacing loop 2, and shown to produce a ribozyme which has lower catalytic efficiency. Another mimetic is a flexible alkyl spacer similar to the polyamide backbone described by Nielsen et al., *Science* **1991**, *254*:1497 (see, Figure 4; **6** or a derivative thereof **6a**; Zuckerman et al., *J. Am. Chem. Soc.* **1992**, *114*:10464). Use of such mimetics allows about 2-10 nucleotides to be omitted from the final nucleic acid molecule compared to the use of an oligonucleotide without a non-nucleotide mimetic.

Example 3: Synthesis of Non-nucleotide Mimetics Aromatic Spacer Phosphoramidite 2

This compound was originally described by Salunkhe et al., *J. Am. Chem. Soc.* **1992**, *114*:6324. The synthesis was modified as follows: To terphthalic acid (1.0 g, 6.0 mmol) in DMF (12 mL) was added EDC (2.54 g, 13.2 mmol), aminohexanol (1.55 g, 13.2 mmol) and *N*-methylmorpholine (1.45 mL, 13.2 mmol). The reaction mixture was stirred overnight at which time the solution was cloudy. Water was added to the reaction mixture to precipitate out the product. The solid was filtered and washed with water and dried to provide 562 mg (25.7%) of the diol.

To the diol (250 mg, 0.687 mmol) in DMSO (40 mL) was added triethylamine (287 μL, 2.06 mmol), dimethoxytrityl chloride (220 mg, 0.653 mmol) and

Compound **4**, R=H, was prepared according to Iyer et al., *Nucleic Acids Res.* **1990**, *18*:2855. Referring to Figure 7, compounds **4** and **5** (R=*O*-*t*-butyldimethylsilyl) phosphoramidites were prepared as follows:

To a solution of *D*-ribose (20.0 g, 0.105 mol) in N,N-dimethylformamide (250 mL) was added 2,2-dimethoxypropane (50 mL) and *p*-toluenesulfonic acid monohydrate (300 mg). The reaction mixture was stirred for 16 hours at room temperature and then evaporated to dryness. The crude product was coevaporated with pyridine (2 x 150 mL), dissolved in dry pyridine (300 mL) and 4,4'-dimethoxytrityl chloride (37.2 g, 0.110 mol) was added and stirred for 24 hours at room temperature. The reaction mixture was diluted with methanol (50 mL) and evaporated to dryness. The residue was dissolved in chloroform (800 mL) and washed with 5% $NaHCO_3$ (2 x 200 mL), brine (300 mL), dried, evaporated, coevaporated with toluene (2 x 100 mL) and purified by flash chromatography in $CHCl_3$ to yield 40.7 g (78.1%) of compound **a**.

To a solution of dimethoxytrityl derivative **a** (9.0 g, 18.3 mmol) and DMAP (4.34 g, 36 mmol) in dry CH_3CN, phenoxythiocarbonyl chloride (3.47 g, 20.1 mmol) was added dropwise under argon. The reaction mixture was left for 16 hours at room temperature, then evaporated to dryness. The resulting residue was dissolved in chloroform (200 mL), washed with 5% $NaHCO_3$, brine, dried, evaporated and purified by flash chromatography in $CHCl_3$, to yield 8.0 g (69.5%) of compound **b** as the β-anomer.

To a solution of intermediate **b** (3.0 g, 4.77 mmol) in toluene (50 mL) was added AIBN (0.82 g, 5.0 mmol) and Bu_3SnH (1.74 g, 6.0 mmol) under argon and the reaction mixture was kept at 80°C for 7 hours. The solution was evaporated and the resulting residue purified by flash chromatography in $CHCl_3$ to yield 1.5 g (66%) of protected ribitol **c**.

Subsequent removal of all protecting groups by acid treatment and tritylation provided the protected

strategy. Such assays will also determine the intracellular localization of the ribozyme following uptake, ultimately establishing the requirements for maintenance of steady-state concentrations within the cellular compartment containing the target sequence (nucleus and/or cytoplasm). Efficacy and cytotoxicity can then be tested. Toxicity will not only include cell viability but also cell function.

Some methods of delivery that may be used include:

a. encapsulation in liposomes,
b. transduction by retroviral vectors,
c. conjugation with cholesterol;
d. localization to nuclear compartment utilizing antigen binding or nuclear targeting site found on most snRNAs or nuclear proteins,
e. neutralization of charge of ribozyme by using nucleotide derivatives, and
f. use of blood stem cells to distribute ribozymes throughout the body.

Delivery strategies useful in the present invention, include: ribozyme modifications, and particle carrier drug delivery vehicles. Unmodified ribozymes, like most small molecules, are taken up by cells, albeit slowly. To enhance cellular uptake, the ribozyme may be modified essentially at random, in ways which reduce its charge but maintains specific functional groups. This results in a molecule which is able to diffuse across the cell membrane, thus removing the permeability barrier.

Modification of ribozymes to reduce charge is just one approach to enhance the cellular uptake of these larger molecules. The random approach, however, is not advisable since ribozymes are structurally and functionally more complex than small drug molecules. The structural requirements necessary to maintain ribozyme catalytic activity are well understood by those in the

hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesive microspheres.

From this category of delivery systems, liposomes are preferred. Liposomes increase intracellular stability, increase uptake efficiency and improve biological activity.

Liposomes are hollow spherical vesicles composed of lipids arranged in a similar fashion as those lipids which make up the cell membrane. They have an internal aqueous space for entrapping water soluble compounds and range in size from 0.05 to several microns in diameter. Several studies have shown that liposomes can deliver RNA to cells and that the RNA remains biologically active.

For example, a liposome delivery vehicle originally designed as a research tool, Lipofectin, has been shown to deliver intact mRNA molecules to cells yielding production of the corresponding protein. In another study, an antibody targeted liposome delivery system containing an RNA molecule 3,500 nucleotides in length and antisense to a structural protein of HIV, inhibited virus proliferation in a sequence specific manner. Not only did the antibody target the liposomes to the infected cells, but it also triggered the internalization of the liposomes by the infected cells. Triggering the endocytosis is useful for viral inhibition. Finally, liposome delivered synthetic ribozymes have been shown to concentrate in the nucleus of H9 (an example of an HIV-sensitive cell) cells and are functional as evidenced by their intracellular cleavage of the sequence. Liposome delivery to other cell types using smaller ribozymes (less than 142 nucleotides in length) exhibit different intracellular localizations.

Liposomes offer several advantages: They are non-toxic and biodegradable in composition; they display long circulation half-lives; and recognition molecules can be readily attached to their surface for targeting to tissues. Finally, cost effective manufacture of liposome-

intraperitoneal, intranasal, intrathecal and ophthalmic. Each of these administration routes expose the ribozyme to an accessible diseased tissue. Subcutaneous administration drains into a localized lymph node which proceeds through the lymphatic network into the circulation. The rate of entry into the circulation has been shown to be a function of molecular weight or size. The use of a liposome or other drug carrier localizes the ribozyme at the lymph node. The ribozyme can be modified to diffuse into the cell, or the liposome can directly participate in the delivery of either the unmodified or modified ribozyme to the cell. This method is particularly useful for treating AIDS using anti-HIV ribozymes.

Also preferred in AIDS therapy is the use of a liposome formulation which can deliver oligonucleotides to lymphocytes and macrophages. This oligonucleotide delivery system inhibits HIV proliferation in infected primary immune cells. Whole blood studies show that the formulation is taken up by 90% of the lymphocytes after 8 hours at 37°C. Preliminary biodistribution and pharmacokinetic studies yielded 70% of the injected dose/gm of tissue in the spleen after one hour following intravenous administration. This formulation offers an excellent delivery vehicle for anti-AIDS ribozymes for two reasons. First, T-helper lymphocytes and macrophages are the primary cells infected by the virus, and second, a subcutaneous administration delivers the ribozymes to the resident HIV-infected lymphocytes and macrophages in the lymph node. The liposomes then exit the lymphatic system, enter the circulation, and accumulate in the spleen, where the ribozyme is delivered to the resident lymphocytes and macrophages.

Intraperitoneal administration also leads to entry into the circulation, with once again, the molecular weight or size of the ribozyme-delivery vehicle complex controlling the rate of entry.

August 6, 1993, hereby incorporated by reference herein in its entirety.

Other embodiments are within the following claims.

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE:	(213) 489-1600
(B) TELEFAX:	(213) 955-0440
(C) TELEX:	67-3510

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	11
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(ix) FEATURE:

(D) OTHER INFORMATION:	The letter "N" stands for any base. "H" represents nucleotide C, A, or U.

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

NNNNUHNNNN N 11

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	32
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

46X

(ix) FEATURE:

(D) OTHER INFORMATION:	The letter "N" stands for any base.

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

NNNNNCUGAN GAGGCCGAAA GGCCGAAANN NN 32

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

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GAGGGGACCG UCCCCUCGGU AAUGGCGAAU GGGAC 85

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	36
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

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(A) LENGTH:	33
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
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(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

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(A) LENGTH:	29
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(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

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(i) SEQUENCE CHARACTERISTICS:

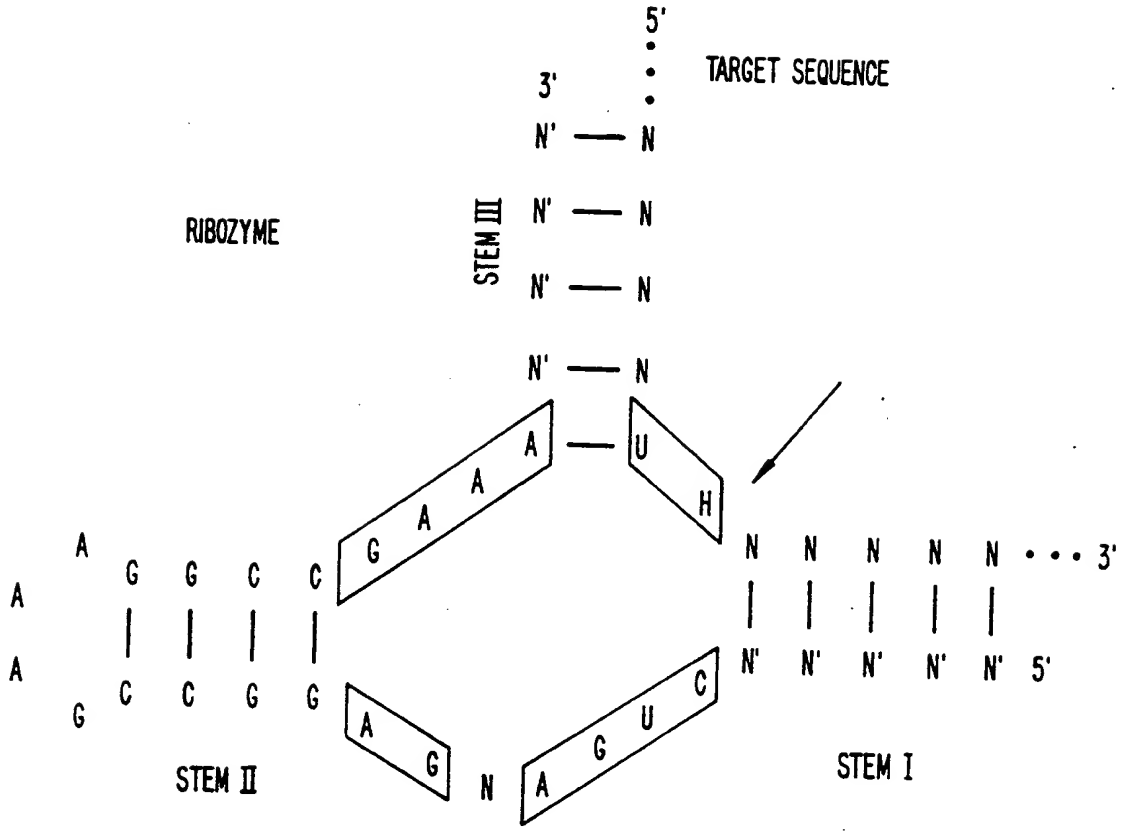
Claims

1. Enzymatic nucleic acid comprising a non-nucleotide.

2. The enzymatic nucleic acid of claim 1, wherein said non-nucleotide is provided in a double-stranded stem region, the catalytic core, or in a single-stranded recognition region.

3. The enzymatic nucleic acid of claim 1, wherein said non-nucleotide is selected from the group consisting of compound 1, 2, 3, 4 and 5.

FIG. 1.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STEM III
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N' — N
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G
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C
C
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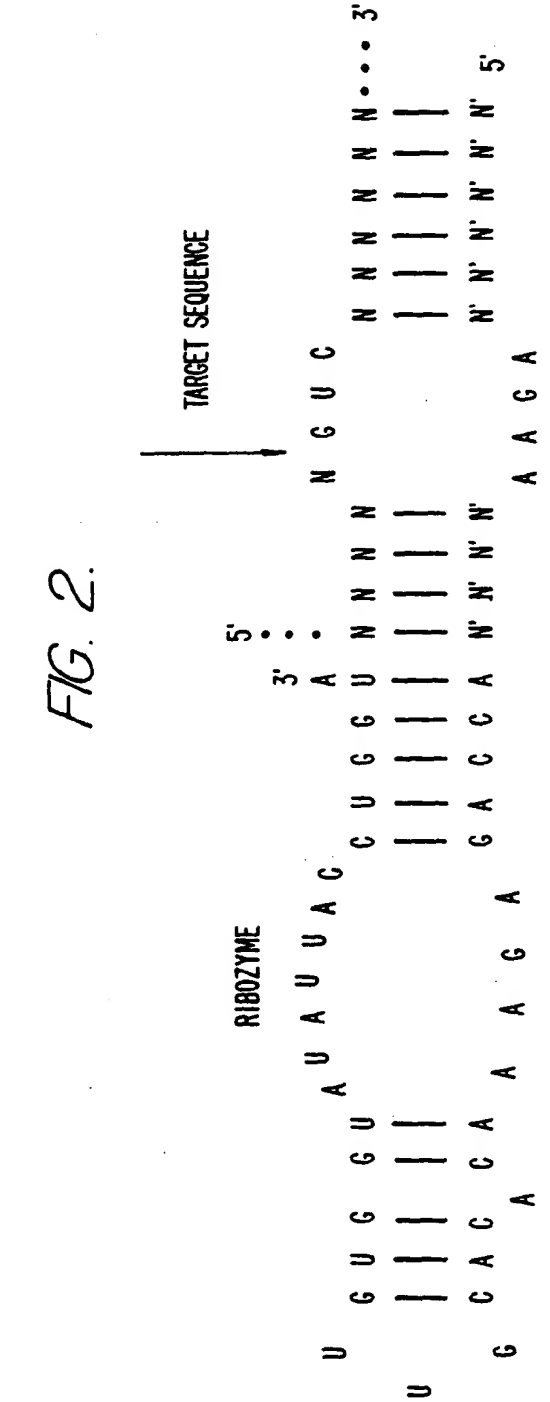
STEM II
STEM I

FIG. 2.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G

FIG. 3.

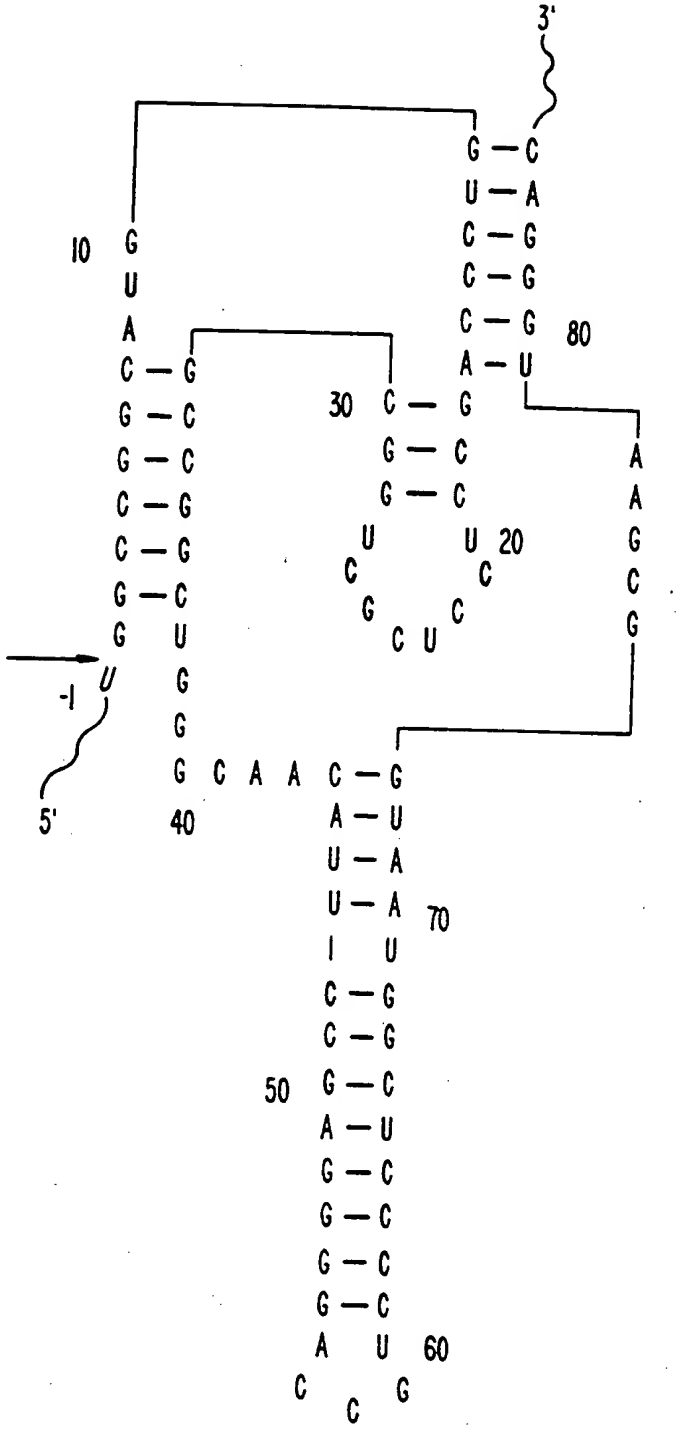


FIG. 4.

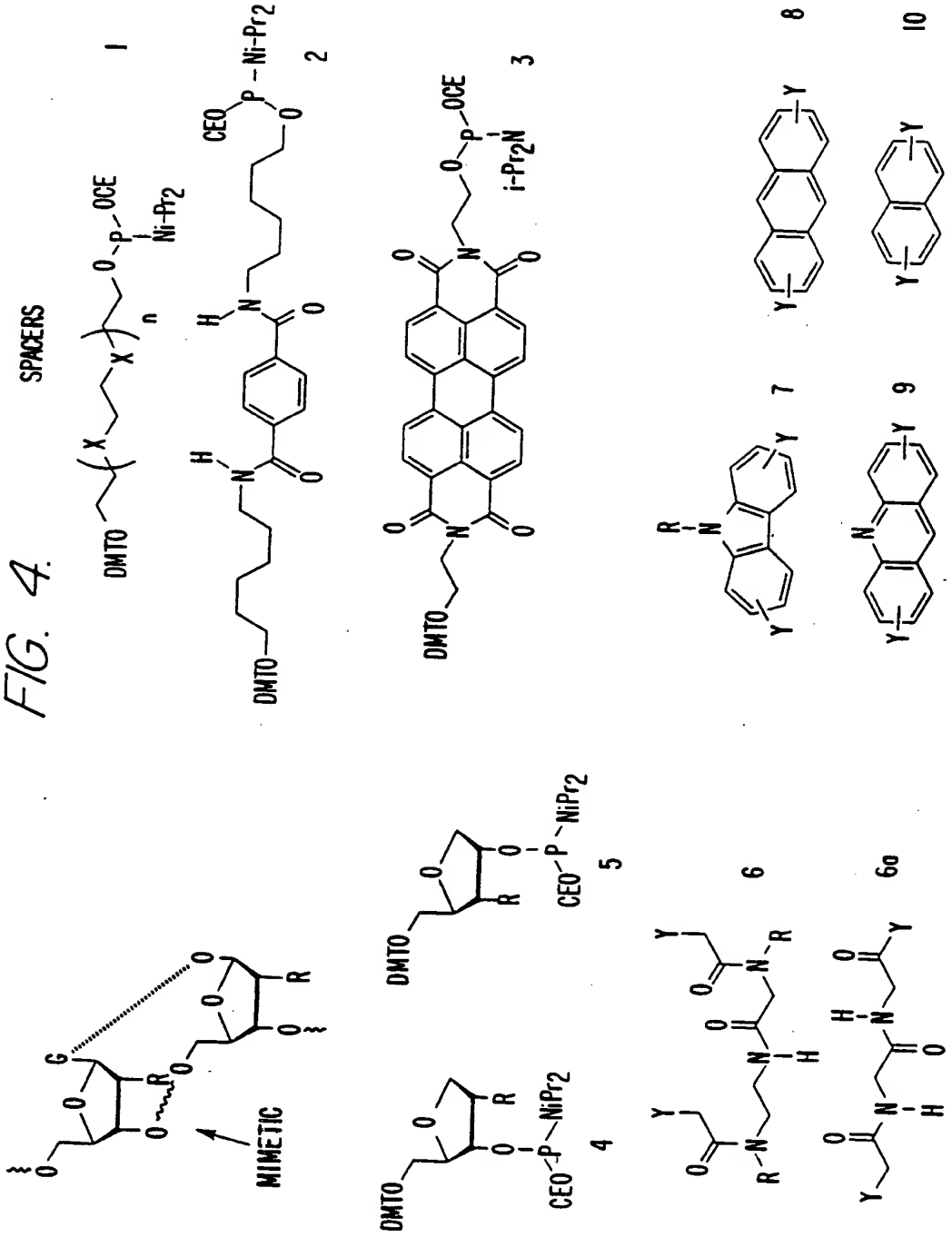
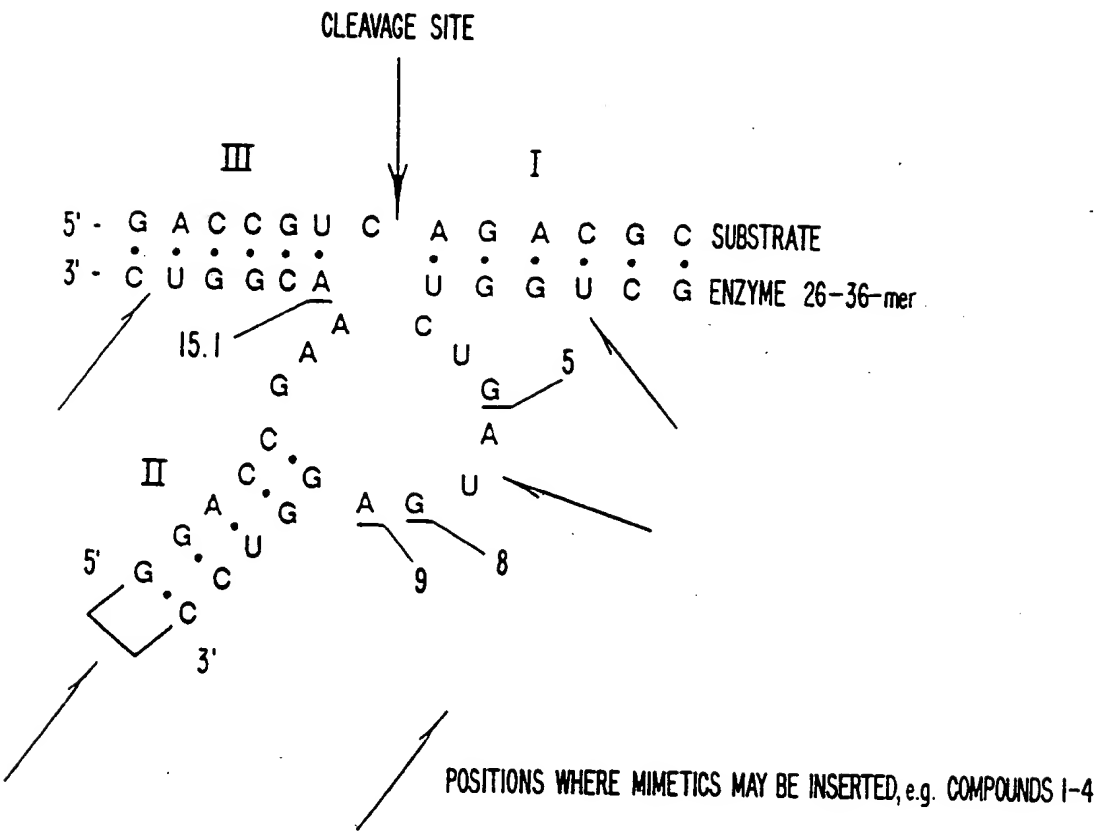


FIG. 5.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A
A
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C
U
G
5
A
C
C
G
II
A
G
U
A G
G
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9
8
POSITIONS WHERE MIMETICS MAY BE INSERTED, e.g. COMPOUNDS 1-4

FIG. 6.

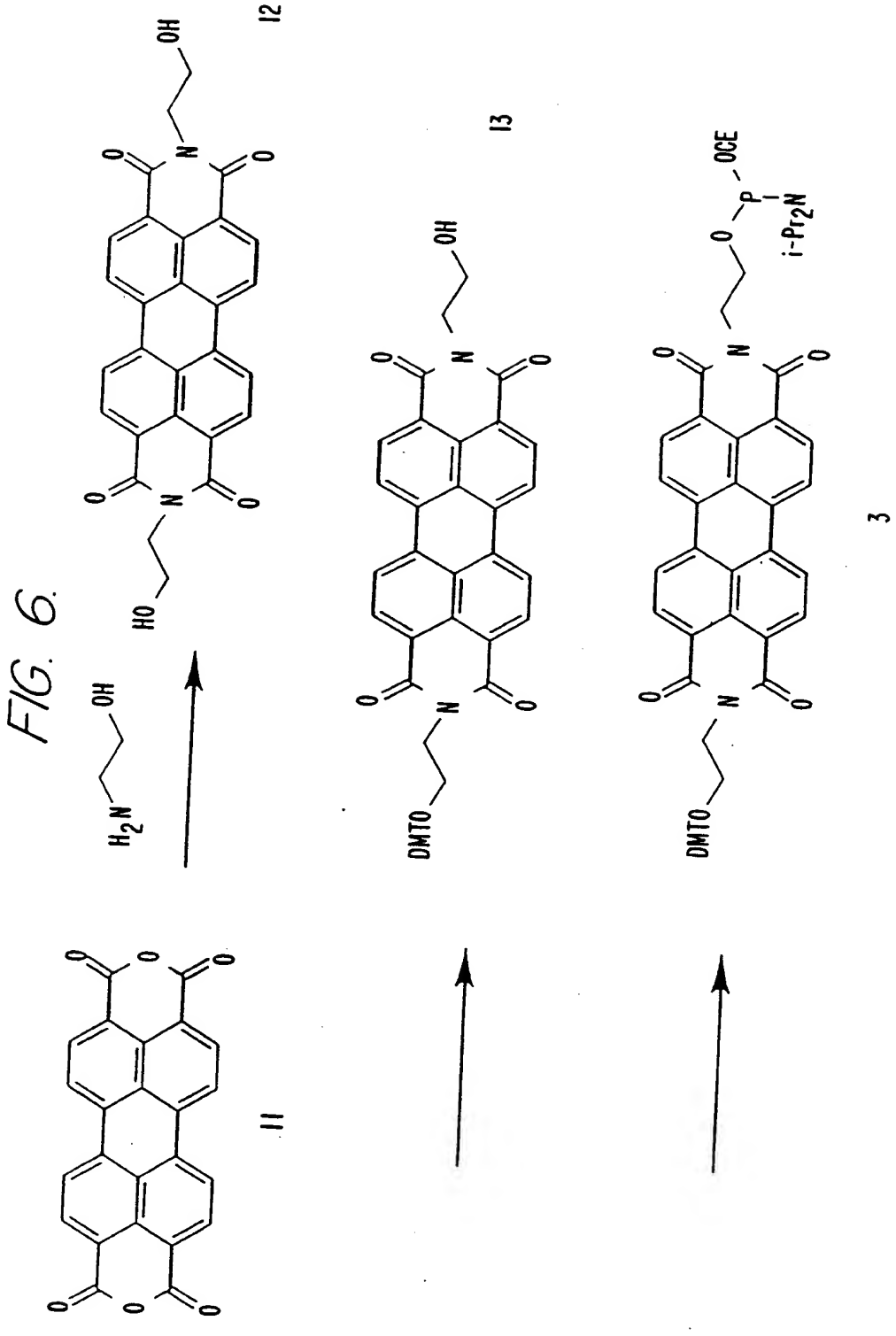


FIG. 7.

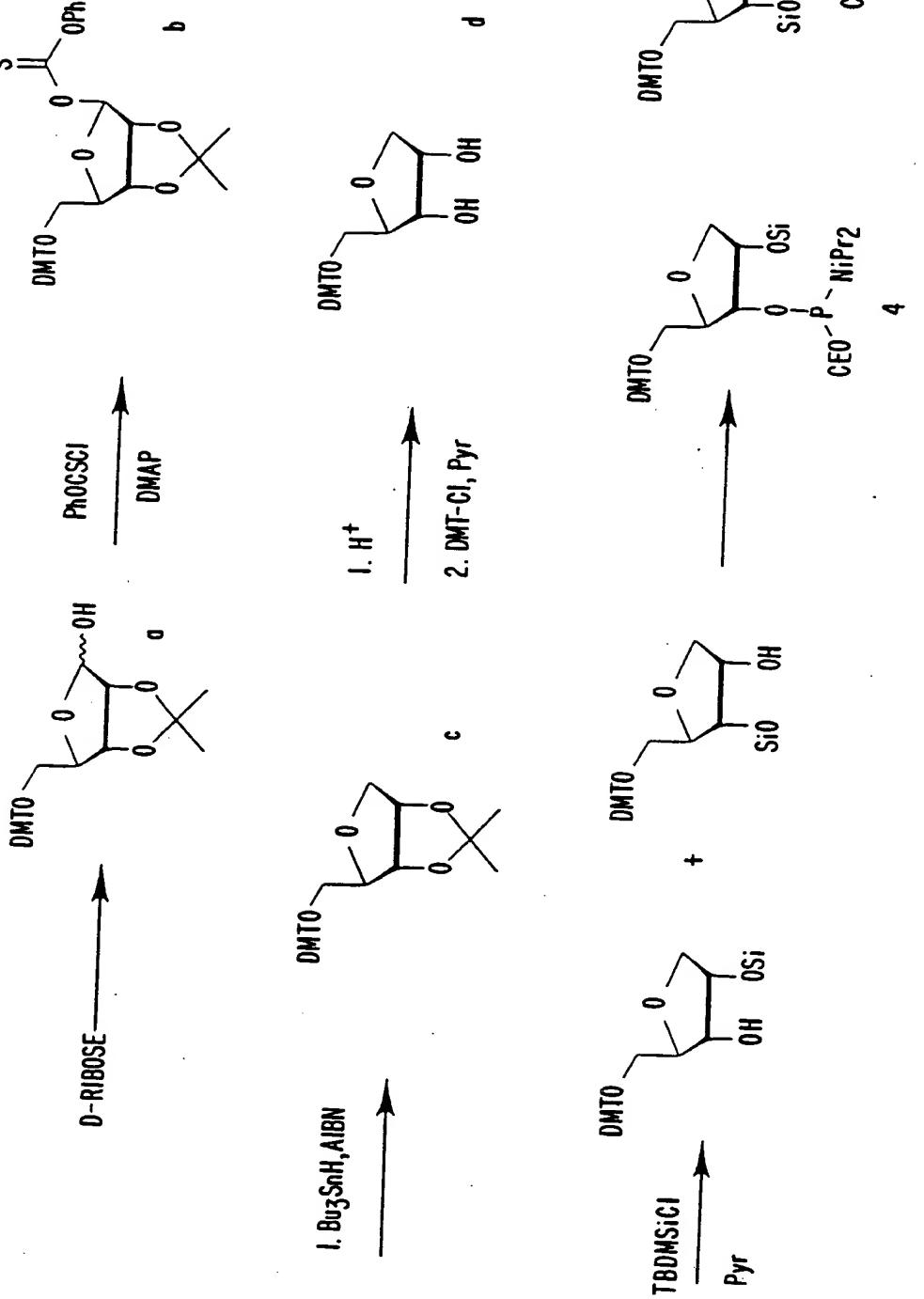
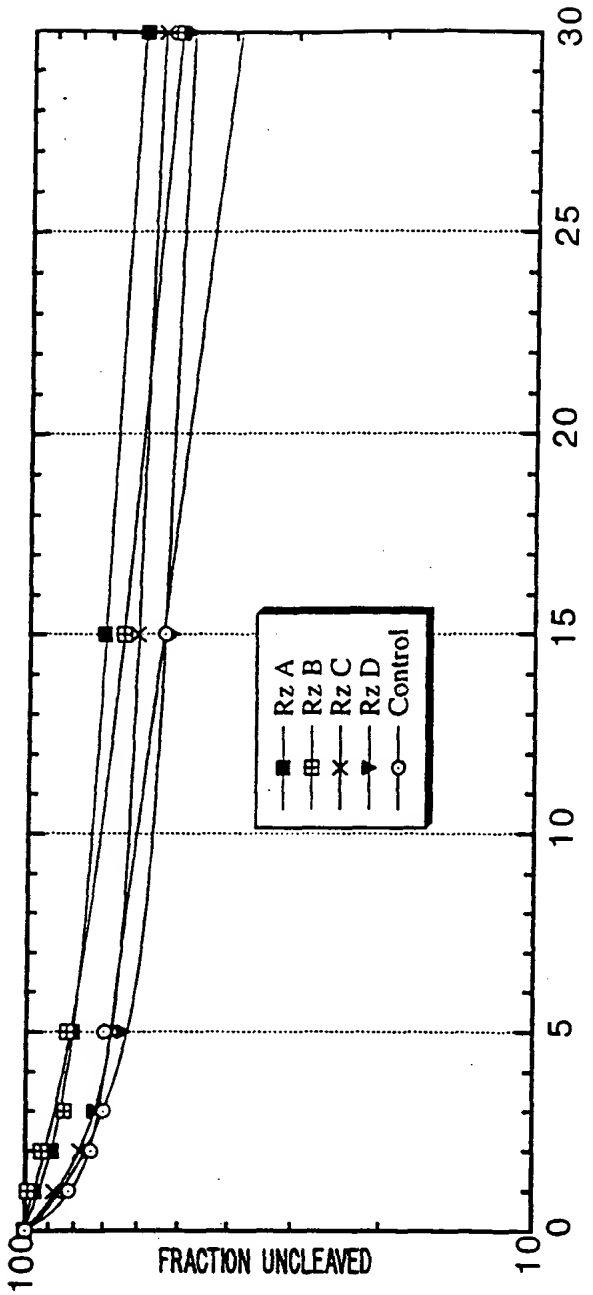

D-RIBOSE
a
PhOCSCl
DMAP
b
1. Bu3SnH, AIBN
c
1. H+
2. DMT-Cl, Pyr
d
TBDMSiCl
Pyr
DMTO
OH
OPh
S
O
OSi
SiO
CEO
P
NiPr2
4
5
+

FIG. 8a



Rz A y = m1*exp(-m2*m0) + m3*exp(...	Value	Error
m1	18.469	3.7629
m2	0.39253	0.1409
m3	82.388	3.7527
m4	0.010447	0.0020647
Chisq	7.4368	NA
R	0.99682	NA

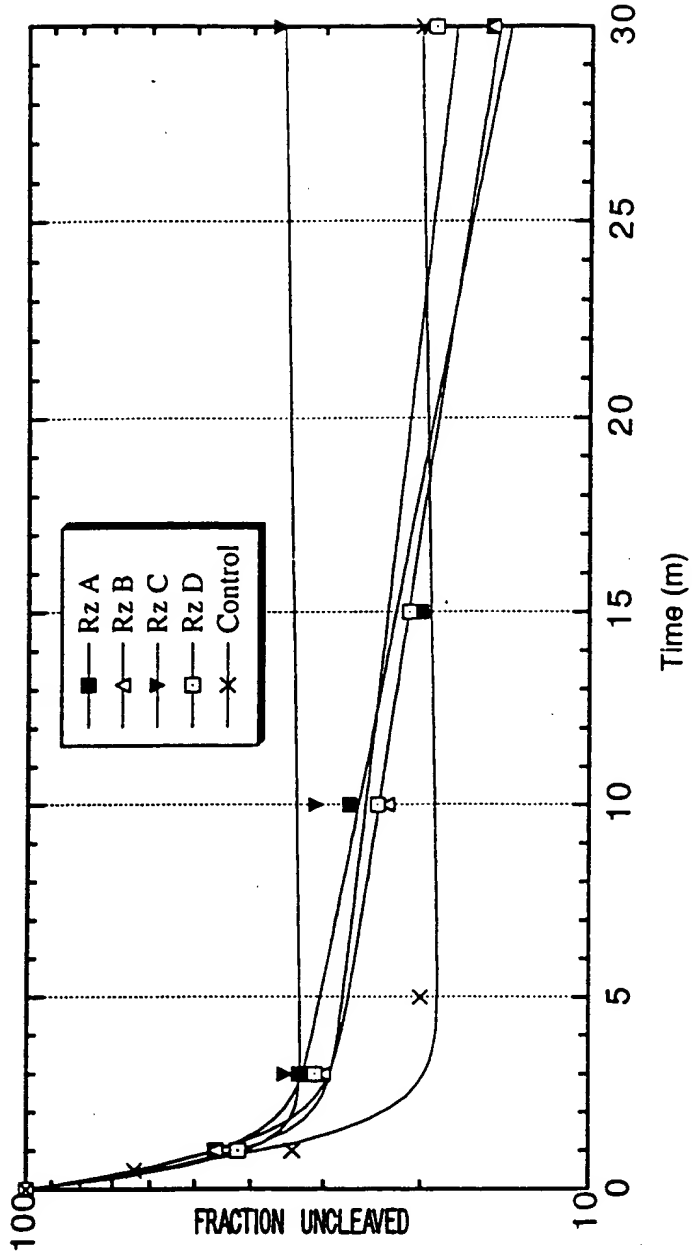
Rz B y = m1*exp(-m2*m0) + m3*exp(...	Value	Error
m1	20.898	14.545
m2	0.21686	0.1902
m3	80.591	14.99
m4	0.015244	0.0072584
Chisq	28.467	NA
R	0.99282	NA

Rz C y = m1*exp(-m2*m0) + m3*exp(...	Value	Error
m1	33.455	1.9896
m2	0.50395	0.060309
m3	66.98	1.9005
m4	0.0065002	0.0013287
Chisq	3.2535	NA
R	0.99888	NA

Rz D y = m1*exp(-m2*m0) + m3*exp(...	Value	Error
m1	38.104	6.7642
m2	0.40912	0.13303
m3	60.267	6.6808
m4	0.0074357	0.0049864
Chisq	27.831	NA
R	0.99255	NA

Control y = m1*exp(-m2*m0) + m3*exp(...	Value	Error
m1	24.205	3.1876
m2	1.2349	0.40326
m3	75.913	2.6166
m4	0.022247	0.0039455
Chisq	7.7965	NA
R	0.99665	NA

FIG. 8b.



Rz A y = m1*exp(-m2*m0) + m3*exp(...	Value	Error
m1	64.468	3.0502
m2	1.7159	0.23412
m3	35.528	2.4005
m4	0.03126	0.0052961
Chisq	7.2589	NA
R	0.99926	NA

Rz B y = m1*exp(-m2*m0) + m3*exp(...	Value	Error
m1	69.397	1.3705
m2	1.45	0.075309
m3	30.585	1.081
m4	0.024723	0.0025193
Chisq	1.4949	NA
R	0.99985	NA

Rz C y = m1*exp(-m2*m0) + m3*exp(...	Value	Error
m1	67.378	4.4664
m2	2.0834	0.5058
m3	32.614	3.0583
m4	-0.0027211	0.0048674
Chisq	10.656	NA
R	0.99841	NA

Rz D y = m1*exp(-m2*m0) + m3*exp(...	Value	Error
m1	69.572	3.5965
m2	1.7304	0.2666
m3	30.408	2.693
m4	0.01864	0.0060488
Chisq	11.594	NA
R	0.99878	NA

Control y = m1*exp(-m2*m0) + m3*exp(...	Value	Error
m1	82.852	10.324
m2	1.4042	0.36303
m3	18.289	8.1056
m4	-0.0032029	0.0199
Chisq	46.535	NA
R	0.99506	NA